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Study on the Effect of Mass Selection and Hybridization on Growth Performance of Chinese Pearl Oyster *Pinctada martensii*

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The pearl oyster *Pinctada martensii* is an important species for sea pearl production in China. To explore the influence of the combination of mass selection and hybridization on growth performance of *P. martensii*, we established four selected groups and four control groups (each with two within-family crosses and two reciprocal hybrid crosses) using 1-year-old offspring of two families (Family A and Family B) from Beihai, Guangxi Province, China. Generally, the growth of the selected group was greater than that of the control group for both within-family crosses and reciprocal hybrid crosses. Shell length and width were affected by genotype, environmental factors, and the interaction between the two on Days 210 and 360. The shell widths of the four reciprocal hybrid crosses all showed heterosis on Day 360. The four within-family crosses showed a certain degree of inbreeding depression during the growth period. On Day 360, the three genetic parameters for shell width of the selected group of ♀ Family B and ♂ Family A were the largest, with values of 0.70, 1.17, and 0.06 for realized heritability, standard response to selection, and current genetic gain, respectively. Overall, the growth performance of ♀ Family B × ♂ Family A in the selected group was the best. Therefore, the combination of mass selection and hybridization could be an effective way to improve the growth performance of *P. martensii*.

Keywords: *Pinctada martensii*, mass selection, hybridization, production performance, response to selection

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INTRODUCTION

Pinctada martensii is a dioecious pearl oyster native to Guangxi, Guangdong, and Hainan provinces in China. The pearls produced by *P. martensii* are called "South China Sea pearl." *P. martensii* is the most important seawater pearl-producing species in China, and it once enjoyed a worldwide reputation. However, in recent years, the output of pearls in China has decreased significantly, and the quality and price of pearls have continued to decline. The germplasm of *P. martensii* in China is degraded seriously, with growth rate, small size, and poor secretion ability (Gu et al., 2009; Liu et al., 2011; Li et al., 2017; He et al., 2021). One of the most important reasons for the problems above is the inability to provide high-quality seedlings for the industry. Therefore, the breeding program of *P. martensii* is imperative. Mass selection is a frequently used and effective

method in shellfish breeding. However, due to the destruction of the natural environment, the wild population resources of *P. martensii* are very scarce. Aquaculture currently mainly relies on hatchery production, while the shellfish in the hatchery can easily cause inbreeding depression due to the long-term inbreeding (Li et al., 2017). Hybridization is an effective method to reduce inbreeding depression. Therefore, the combination of mass selection and crossbreeding cannot only quickly achieve the purpose of breeding, but also reduce the decline of inbreeding.

Due to poorly developed selective breeding technology, the global output of aquatic products after selective breeding only accounted for 8.2% of the total aquaculture in 2010 (Gjedrem and Rye, 2018). The genetic gain of each generation of selected aquatic species can reach 12.5%, and the annual growth rate can reach 5.4%. If selective breeding is applied to all aquatic species, the world aquaculture production could be doubled in 13 years (Gjedrem et al., 2012). Effective selective breeding requires selection of parents with favorable phenotypes, such as fast growth and strong stress resistance (Gjedrem and Baranski, 2009). To date, many shellfish selective breeding programs initially selected parents from wild populations (Li et al., 2011; Zhao et al., 2012; Dégremont et al., 2015; Du et al., 2015; He, 2016; Barros et al., 2018), which have high genetic diversity and are easy to adapt to the environment due to natural selection (Zhao et al., 2020). However, some shellfish breeding programs have used advantageous breeding groups that are selected from laboratory strains as the basic populations (Li, 2012; Huo et al., 2015; Wang et al., 2020).

Mass selection and hybridization are common and effective approaches that have been widely used for genetic improvement of aquaculture animals. The advantage of mass selection for highly fertile aquatic animals is that strong selection pressures can be applied to them (Gjedrem and Baranski, 2010). Results from challenge test experiments on animals from family selection can be highly consistent with results from natural experiments, but the former are more expensive to conduct (Gjedrem and Rye, 2018). Mass selection is also a lower-cost alternative to family selection (Dégremont et al., 2015). Dégremont et al. (2015) reported that after four generations of mass selection for the Pacific oyster *Crassostrea gigas*, resistance to the ostreid herpesvirus 1 and the growth rate of each generation improved gradually. However, the growth rate of offspring selected from a suitable environment will not show a significant advantage compared with the control group in an unsuitable environment (Deng et al., 2009a; Zhao et al., 2019, 2020). Crossbreeding, which uses dominant parents to produce offspring with heterosis (Newkirk, 1980), is one method that may improve profitable traits. Crosses between inbred lines can also reduce the increased inbreeding coefficient of multiple generations of inbreeding and at the same time increase genetic diversity. Huo et al. (2015) selected two families with the fastest growth rate from 45 full-sib families of the clam *Ruditapes philippinarum* and applied a certain intensity of selection to self-crosses and crosses. The hybrid offspring showed obvious signs on growth at the age of 30–90 days. Thus, the growth advantage conferred by increasing the selection intensity of highly fertile species followed by hybridization may improve production performance. Researchers have carried out a series of breeding programs on *P. martensii*, including mass selection (Deng et al., 2009b; Wang et al., 2011), cross breeding (Gu et al., 2011), and molecular marker assisted breeding (Shi et al., 2009). Through these studies, new varieties of *P. martensii* with genetic advantages, such as "Haixuan No. 1" (Du et al., 2015), "Haiyou No. 1" (Li, 2012), and "Nanke No. 1" (He, 2016), have been cultivated. These new varieties have the characteristics of fast growth, large shell width, and a strong secreting ability.

In this study, we combined mass selection and hybridization to evaluate the growth parameters of the selected groups and the control groups, as well as the inbred and hybrid groups. We calculated the heterosis and inbreeding depression based on measurements of shell length and width and estimated values for several genetic parameters for shell width of the selected groups.

MATERIALS AND METHODS

Base Stock and Intensity of Selection

In March 2019, we established 27 families using three new varieties of *P. martensii* (Haiyou No. 1, Haixuan No. 1, and Nanke No. 1). In April 2020, the 1-year-old offspring of these 27 families were transported from the culture sites to Guangxi Comprehensive Test Station Hatchery of the National Shellfish Industry Technology System at Guangxi Academy of Fisheries Sciences. The shell width of specimens from the 27 families was measured using a vernier caliper (accuracy 0.01 mm), and the two families (named Family A and Family B) with the largest mean shell width were selected as the parent population for our experiment. The parents of Family A were ♀ Haixuan No. 1 and ♂ Nanke No. 1, and the parents of Family B were ♀ Nanke No. 1 and ♂ Haiyou No. 1. Seven bags of offspring were randomly selected for each family. After removing the dead individuals and those with irregularly shaped shells, there were 1,075 individuals in Family A and 1,025 individuals in Family B. We used a vernier caliper (accuracy 0.01 mm) to measure the shell width of all individuals from the two families, and then the top 12% of individuals with the largest shell width from each family were selected as the selected groups (named SA and SB, respectively). Additionally, 100 individuals were randomly taken from each family to serve as the control groups (named CA and CB, respectively) before selection. Due to different levels of gonadal development, we actually used 112 individuals from Family A and 120 individuals from family B during the experiment. **Figure 1** and **Table 1** show the shell width frequency distribution, selection cutoff points, and intensity of selection (*i*) of the two families.

Establishment of Crosses

Oocytes from females were obtained by dissecting gonads and gathering them into a 50 L bucket filled with filtered sea water. Impurities such as tissue fragments were filtered out with a 100 μm mesh screen. For each line, egg suspensions were mixed well and divided equally into six 10 L buckets. A few drops of ammonia (2–3 mM) (Ohta et al., 2007) were added and the

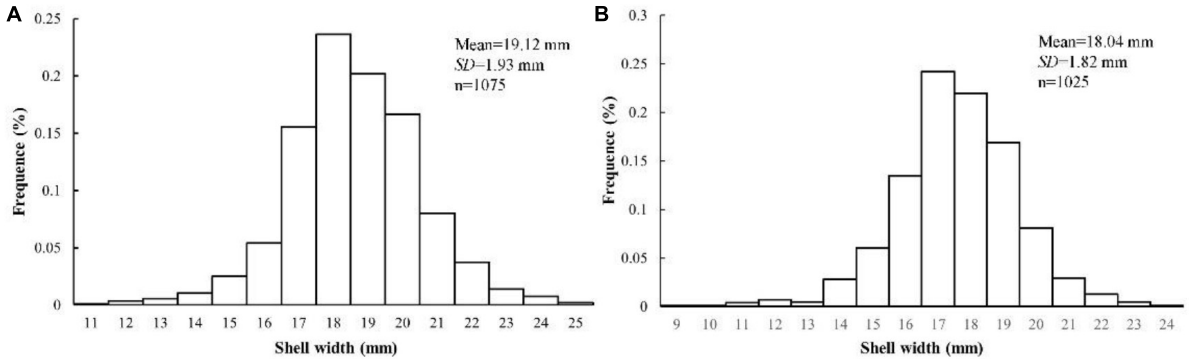

FIGURE 1 | Shell width frequency distribution of *P. martensii*: **(A)** Family A and **(B)** Family B.

TABLE 1 | Shell width (mean ± SD) of base populations and selected group parents, cutoff points, and selection intensity of *P. martensii.*

Items	Base population shell width (mm)	Cutoff point (mm)	Selected parents			Selection intensity
			Sire	Dam	Shell width (mm)	
Family A	19.12 ± 1.93	22.59	61	51	22.43 ± 1.01	1.72
Family B	18.04 ± 1.82	20.05	68	52	20.99 ± 0.91	1.62

TABLE 2 | Mating strategy of *P. martensii.*

Parents	♀ SA	♀ SB	♀ CA	♀ CB
♂SA	SAA	SBA	—	—
♂SB	SAB	SBB	—	—
♂CA	—	—	CAA	CBA
♂CB	—	—	CAB	CBB

buckets were set aside. Oocyte maturity was monitored during the process of oocytes soaking. Semen was extracted from the gonad of each male with a pipette and then divided equally into six 1 L beakers. According to Qin et al. (2018), after the germinal vesicle breakdown ratio of the oocytes reached 100%, a few drops of ammonia were added to the seminal fluid, and the sperm activity was observed immediately. Eggs were examined to rule out the occurrence of uncontrolled fertilization before formal fertilization. When we observed that most of the sperm were activated, an appropriate amount of seminal fluid was poured into the oocyte fluid for fertilization to produce two within-family crosses and two reciprocal hybrid crosses for both the selected groups and the control groups. **Table 2** shows the mating strategy, and the experiment was conducted in triplicate for each group. The fertilized eggs were pooled and placed in a 1 m^3 beaker for hatching at a density of 10–20 eggs mL^{-1}. The temperature of the hatching water was maintained at 28°C, and the salinity was kept at 30 ppt.

Larval Rearing, Spat Nursery, and Grow-Out

The fertilized eggs developed into D-veliger larvae in about 16 h. Larvae were collected after being passed through a 40 μm nylon screen, and the density of each group was adjusted to 3–5 individuals mL^{-1}. The density of each group was adjusted regularly during cultivation to eliminate the effect of density. The larvae were fed on *Isochrysis galbana* before they reached a size of 110–120 μm. As the spat grew, *Chaetoceros muelleri* and *Platymonas subcordiformis* were added to the diet. Feeding was gradually increased from 3,000 to 50,000 cells mL^{-1} day^{-1}. The proportion of the three phytoplankton species was 1:1:1. The water was completely exchanged with filtered seawater once a day.

The spat from each group were gathered into polyethylene mesh bags with a 2 mm aperture at a density of 120–150 individuals/bag when the shell length of juveniles was about 2–3 mm. They were then transferred to three culture sites in Beihai in Guangxi Zhuang Autonomous Region. The three culture sites were named Zhulin (Z), Huolu (H), and Qingshantou (Q) (**Figures 2a,b**). The mesh bags at Zhulin were hung on a floating raft in a large pond (**Figure 2c**). The mesh bags at Huolu and Qingshantou were suspended from off-bottom piles, which were located in the shoal offshore (**Figure 2d**). The biofouling on the mesh bags was removed regularly to prevent it from affecting the flow of seawater through the bags. The mesh bags were changed periodically as the oysters grew. The density of oysters in the mesh bags was adjusted to avoid the influence of density on the experiment.

Evaluation of Growth Performance

The shell length of oysters in each cross was measured on Days 9, 45, 210, and 360, and the shell width was measured on Days 210 and 400. The shell length on Day 9 was measured with a micrometer under a microscope (10×), and the other measurements were made using an electronic vernier caliper (accuracy 0.01 mm).

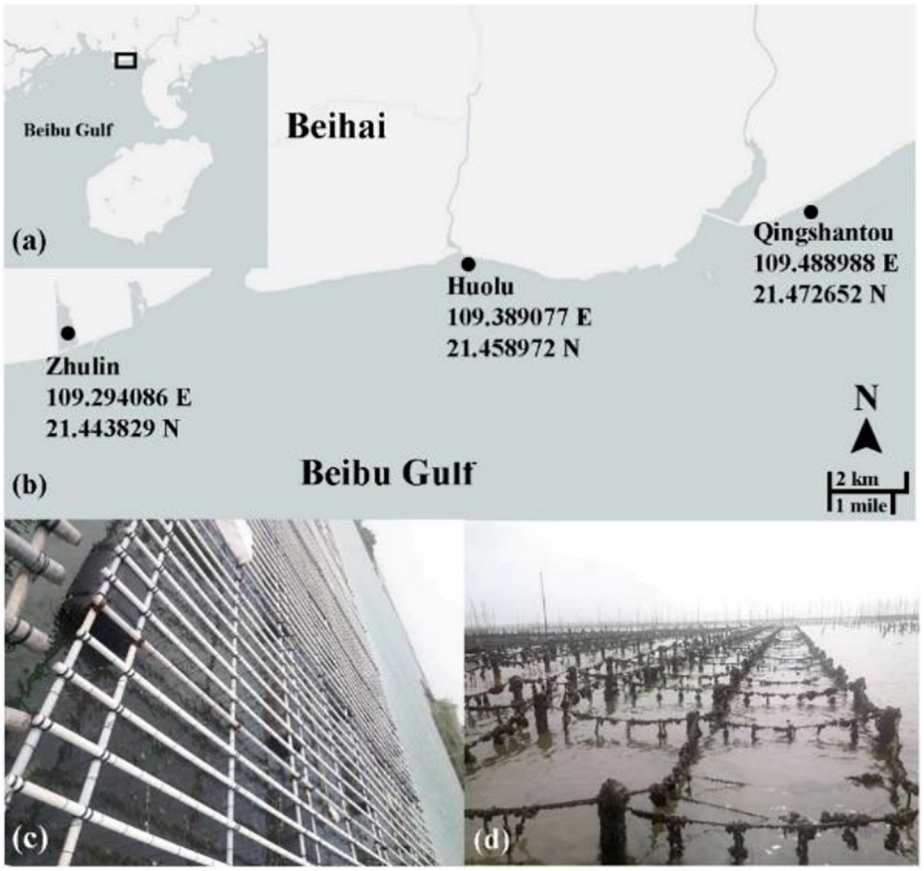

FIGURE 2 | Culture sites and methods. **(a,b)** The three culture sites used in this study; **(c)** oyster hanging culture at Zhulin; **(d)** oyster off-bottom cultures at Huolu and Qingshantou.

Statistical Analyses

Differences in the growth parameters on Days 9 and 45 among the different groups were analyzed by multiple comparisons using a one-way analysis of variance of the means. The growth parameters on Days 210 and 360 among the different groups were analyzed by multiple comparisons using a two-way analysis of variance of the means. The shell length and shell width were log transformed to ensure normality and homoscedasticity. All statistical analyses were performed using R software Version 3.6.3 for Windows. $P < 0.05$ was considered to be statistically significant.

To evaluate the effects of the genotype and environmental factors on the growth of *P. martensii*, a two-factor analysis of variance was used as Zhang et al. (2007):

$$Y_{ijk} = \mu + G_i + E_j + (G \times E)_{ij} + e_{ijk}$$

where Y_{ijk} is the mean shell length or shell width at Days 210 and 360 of the k replicates, i genotype, and j site; G_i is the genotype effect on the mean shell length or shell width at Days 210 and 360 ($i = 1, 2$); E_j is the environmental effect on the mean shell length or shell width at Days 150, 210, and 360 ($j = 1, 2, 3$); $(G \times E)_{ij}$ is the interaction effect between the genotype and the environmental factors; and e_{ijk} is the random observation error ($k = 1, 2, 3$).

Heterosis (H%) was calculated using the following formula (Eq. 1):

$$H\% = \frac{(F_1 - M_p)}{M_p} \times 100 \quad (1)$$

where F_1 is the mean shell length (shell width) of the reciprocal hybrid crosses, and M_P is the mean shell length (shell width) of within-family crosses.

According to Falconer and Mackay (1996), the intensity of selection was calculated as the difference in mean shell width between the selected parents and the base population divided by the standard deviation of the population. The realized heritability ($h^2{}_R$) was calculated following as Hadley et al. (1991):

$$h^2_{\mathrm{R}} = \frac{X_s - X_c}{i\sigma_c} \quad (2)$$

According to Wang (2017), Eq. 2 was adjusted to Eq. 3 to calculate the realized heritability of the reciprocal hybrid crosses for a selected group:

$$h^2_{\mathrm{R}} = \frac{X_s - X_c}{\frac{1}{2}(i_1 + i_2)\sigma_c} \quad (3)$$

where X_S and X_C are the mean shell width of offspring in selected and control groups, respectively; σ_c is the standard deviation of control offspring; and i is the intensity of selection (i_1 for Family A and i_2 for Family B). The standard response to selection (SR) was estimated as Zheng et al. (2006):

$$SR = \frac{X_s - X_c}{\sigma_c} \quad (4)$$

Current genetic gain (GG) was calculated following Zheng et al. (2006):

$$GG(\%) = \frac{X_s - X_c}{X_c} \times 100 \quad (5)$$

The magnitude of inbreeding depression (*ID*) was calculated using the following equation (Zhang et al., 2020):

$$\delta_X\% = \frac{(P_X - S_X)}{P_X} \times 100 \quad (6)$$

where δ_X is the estimate of *ID* for family X; S_X is the mean phenotypic value of offspring from within-family crosses; and P_X is the mean phenotypic value of offspring from reciprocal hybrid crosses.

RESULTS

Growth Parameters of Different Groups

Figure 3 show the growth of shell length and shell width, respectively, in the different groups. The growth rates of each group differed, and the differences began to appear during the larval stage (Day 9). On Day 9, the shell length of the SAB group was the largest at 103.41 ± 9.95 μm, which was significantly larger than that of the other groups ($P < 0.05$) (**Figure 3A**). On Day 45, the shell length of the SAA group was the largest, followed by that of the CAA group. The difference between these two groups was not statistically significant ($P > 0.05$), but their values were significantly higher than those of the other groups ($P < 0.05$) (**Figure 3B**).

During the grow-out stage, both shell length and width were significantly affected by genotype, environmental factors, and the interaction of genotype and environmental factors (**Table 3**). Among the three sites, the shell length of each group was smallest at Zhulin, and the shell lengths of the SBA group on Days 210 and 360 were the largest among the groups within a given site (**Figure 3C**). The shell width of each group was the smallest at Zhulin on Days 210 and 360. With the exception of the SAB group at Qingshantou on Day 210, which had a smaller shell width than that of the CAB group, shell widths were always larger in selected groups than in the control groups. On Day 210, the groups with the largest shell widths at Zhulin, Huolu, and Qingshantou were SBA (9.15 ± 1.69 μm), SAA (11.74 ± 2.03 μm), and SBB (12.71 μm), respectively. On Day 360, the shell width of the SBA group was the largest at all three sites (**Figure 3D**).

Heterosis

Table 4 shows the heterosis results of shell length and shell width for each cross. In general, from the larval stage to the grow-out stage, the heterosis of the selected groups first decreased and then increased, whereas the control groups decreased, increased, and then decreased again. Shell length did not show heterosis in the CAB group ($H\%$ = –0.26%), but all of the other crosses exhibited heterosis at the end of the experiment. The shell width of each cross showed heterosis on Day 360, and the SBA group had the largest value ($H\%$ = 26.12%).

Inbreeding Depression

Table 5 presents the results of *ID* analysis. During the entire growth period, the growth of the four within-family crosses showed varying degrees of *ID*. Values ranged from 6.01 to 7.18% in the larval stage (Day 9), but none of the four within-family crosses showed *ID* by the juvenile stage (Day 45). On Day 210, the shell lengths of the two selected groups exhibited *ID* values of 2.76% (SAA) and 5.16% (SBB). The shell widths of all but the SBB group showed *ID*. At the end of the experiment (Day 360), the *ID* rates for shell length (6.37%) and width (6.61%) were largest in the SBB group.

Shell Width Genetic Parameters

Table 6 shows the realized heritability, standard response to selection, and current genetic gain values for shell width for the four selected groups during the grow-out stage (Days 210 and 360). The three genetic parameters for the reciprocal hybrid crosses all increased, whereas they decreased for the within-family crosses. On Day 210, the values of the three genetic parameters were highest in the SAA group and lowest in the SAB group, whereas on Day 360 the values were highest in the SBA group and lowest in the SAA group.

DISCUSSION

According to New (1991), aquaculture production may have to increase to 63 million tons in 2025 to meet the expected demands. The future needs for aquatic products cannot be met without a substantial increase in aquaculture production because the harvest of many wild stocks has come close to or exceeded the limits of sustainable exploitation (Bentsen and Olesen, 2002). Genetic improvement and domestication are proven routes to increasing agricultural productivity (Hedgecock, 2011). The results of our study indicated that the shell length and width of almost all selected groups were greater than those of the corresponding control groups from the larval stage (Day 9) to the adult stage (Day 360), regardless of mating method or culture site, but the differences were not all statistically significant. The initial selective trait in this study was shell width, so the shell length advantage of the selected groups may be due to the strong correlation between shell length and width of *P. martensii*. Additionally, the operation methods were the same throughout the experiment at a given site, which suggests that the differences in growth between the selected groups and the control groups were caused by genetics (changes in gene frequency caused by selection).

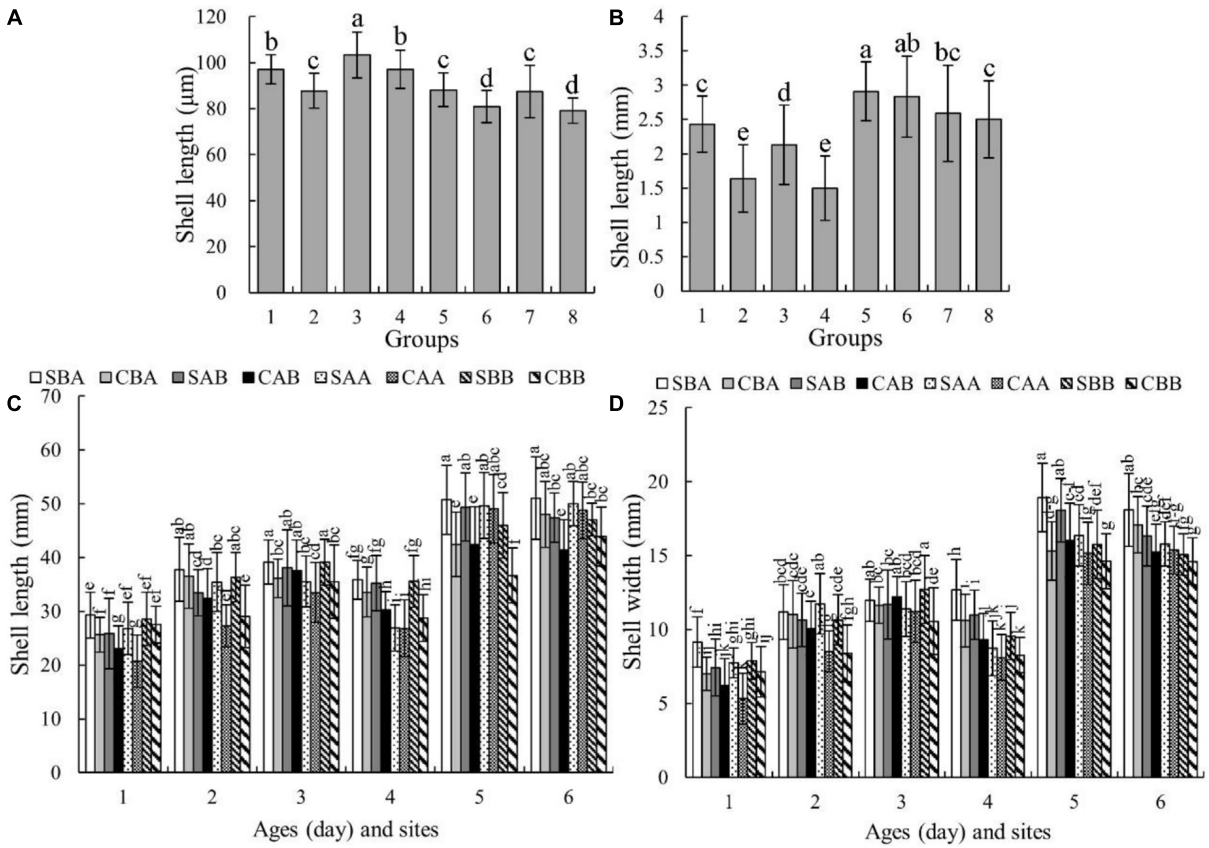

FIGURE 3 | The shell length and shell width of each group of *P. martensii* at different ages and sites. The different letters at each age indicate significant differences among groups ($P < 0.05$). **(A)** Shell length on Day 9, on the horizontal, 1 for SBA, 2 for CBA, 3 for SAB, 4 for CAB, 5 for SAA, 6 for CAA, 7 for SBB, 8 for CBB; **(B)** shell length on Day 45, on the horizontal, 1 for SBA, 2 for CBA, 3 for SAB, 4 for CAB, 5 for SAA, 6 for CAA, 7 for SBB, 8 for CBB; **(C)** shell length on Day 210 and 360, on the horizontal, 1 for Day 210 at Zhulin site, 2 for Day 210 at Huolu site, 3 for Day 210 at Qingshantou site, 4 for Day 360 at Zhulin site, 5 for Day 360 at Huolu site, 6 for Day 360 at Qingshantou site; **(D)** shell width on Day 210 and 360, on the horizontal, 1 for Day 210 at Zhulin site, 2 for Day 210 at Huolu site, 3 for Day 210 at Qingshantou site, 4 for Day 360 at Zhulin site, 5 for Day 360 at Huolu site, 6 for Day 360 at Qingshantou site.

TABLE 3 | One-way analysis of variance showing the differences in growth parameters among different groups (Days 9 and 45) of *P. martensii*.

Group	Sources	*df*	Shell length			Shell width		
			MS	*F*	*P*	*MS*	*F*	*P*
Day 9		7	2123.456	32.333	<0.001***			
Day 45		7	8.165	28.560	<0.001***			
Day 210	G	7	453.860	17.448	<0.001***	63.184	19.806	<0.001***
	E	2	7481.858	287.629	<0.001***	1231.346	385.980	<0.001***
	G × E	14	168.838	6.491	<0.001***	16.635	5.214	<0.001***
Day 360	G	7	696.755	23.802	<0.001***	155.362	43.328	<0.001***
	E	2	17802.111	608.132	<0.001***	3209.583	895.102	<0.001***
	G × E	14	389.451	13.304	<0.001***	11.977	3.340	<0.001***

*Two-way analysis of variance showing the effects of genotype (G) and environmental factors (E) on growth (Days 210 and 360). ***Indicates $P < 0.001$.*

When planning a selective breeding program, the genotype-environment interaction is one of the important factors that must be considered. The selection led to changes in gene frequency, which in turn led to changes in genotypes, and different genotypes had different sensitivity to the environment. Therefore, the interaction may be related to the sensitivity of different genotypes to the environment (Falconer and Mackay, 1996). Genotype-environment interactions are common in shellfish culture, which may reflect poor control of the environment by shellfish (Evans and Langdon, 2006). Thus, in future breeding projects, we can choose genotypes that are less sensitive to the environment and/or we can breed strains that adapt to

TABLE 4 | Heterosis values for growth of reciprocal hybrid crosses of *P. martensii* (SL: shell length; SW: shell width).

Group	Day 9 SL (%)	Day 45 SL (%)	Day 210 SL (%)	Day 360 SL (%)	Day 210 SW (%)	Day 360 SW (%)
SBA	10.96	−8.96	6.55	9.44	6.75	26.12
SAB	18.38	−20.20	−2.32	6.54	−3.17	16.59
CBA	21.69	−42.31	8.51	7.27	13.50	8.59
CAB	9.79	−36.68	15.68	−0.26	19.53	13.97

TABLE 5 | Inbreeding depression of growth for within-family crosses of *P. martensii* (SL: shell length; SW: shell width).

Group	Day 9 SL (%)	Day 45 SL (%)	Day 210 SL (%)	Day 360 SL (%)	Day 210 SW (%)	Day 360 SW (%)
SAA	6.01	−13.68	2.76	−0.54	0.47	1.20
SBB	6.42	−6.75	5.16	6.37	−0.01	6.61
CAA	6.22	−40.33	−8.77	−2.41	7.42	−1.69
CBB	7.18	−29.80	−2.39	3.94	3.97	2.54

specific environments. In this study, the shell length and width of *P. martensii* were significantly affected by genotype-environment interaction. We found that the growth of each group at the Zhulin site at each time point was the slowest. The Huolu site and Qingshantou site were more suitable for pearl oyster aquaculture than the Zhulin site. This may be due to the low frequency of seawater exchange and the deposition of harmful substances, which make this site unsuitable for the rapid growth of *P. martensii*.

Throughout the experimental period, only spat at the juvenile stage (Day 45) did not show heterosis, whereas other stages exhibited a certain degree of heterosis. On Day 360, all reciprocal hybrid crosses except for the CAB group showed heterosis for shell length. The emergence of heterosis indicated a difference in gene frequency between the two families (Zhang et al., 2017). Maintaining two or more populations with different genetic bases as parents for reciprocal crosses is considered to be a good choice for selective breeding, as it may produce heterosis (Goyard et al., 2008). The parents used in this study were the F_1 generation of two families that are characterized by rapid shell width growth. The parents of the two families were ♀ Haixuan No. 1 × ♂ Nanke No. 1 and ♀ Nanke No. 1 × ♂ Haiyou No. 1. These three new *P. martensii* varieties were generated through multiple generations of breeding. Although they are more suitable for artificial breeding than other populations and have the advantageous traits that aquaculturists expect, they may have lost most of the allelic variability available in the wild. Nevertheless, when multiple new varieties are used to generate crosses with each other, the F_1 generation is guaranteed to be hybrid because the parents are unrelated. We have already observed heterosis (unpublished data) during the growth of the offspring of these two families. However, because the offspring of each family were produced by a pair of parents, the simple use of the dominant family for mass selection may cause *ID*. Therefore, we first selected offspring from the two families and then used the selected population to cross and observe the growth of the hybrid offspring. Ultimately, the target traits of the two families were the same, and whether their offspring will show reduced diversity as a result remains to be determined.

TABLE 6 | Realized heritability (h^R_2), standardized response to selection (SR), and current genetic gain (GG) of selected groups of *P. martensii*.

Group	210			360		
	h^2_R	SR	GG (%)	h^2_R	SR	GG (%)
SBA	0.45	0.76	11.61	0.70	1.17	16.35
SAB	0.12	0.20	7.92	0.46	0.77	12.60
SAA	0.74	1.27	28.13	0.24	0.42	6.18
SBB	0.52	0.85	19.16	0.41	0.66	8.79

Inbreeding is harmful to most organisms and leads to a reduction in fitness (Falconer, 1989). According to Evans et al. (2004), any amount of inbreeding may cause phenotypic *ID*. In our study, the within-family crosses in both the selection and control groups showed *ID*. During the entire experimental period, most of reciprocal hybrid crosses performed better than all within-family crosses. This result showed that although we exerted great intensity of selection on pearl oysters, *ID* is likely to be one factor that affects the growth performance of within-family crosses. *ID* can be explained by the hypotheses of partial dominance and over dominance (Kristensen and Sørensen, 2005; Zheng et al., 2008, 2012). Deleterious recessive effects are also thought to be a major reason for *ID* (Charlesworth and Charlesworth, 1999). *ID* is often more obvious in the early stages of animal development and relatively weak in the later stages (Bierne et al., 1998; McCune et al., 2002; Escobar et al., 2008; Anderson and Hedgecock, 2010; Plough and Hedgecock, 2011). This may be because harmful genes are eliminated with the death of individuals in the early stages of development. This phenomenon is manifested in organisms with high fertility. In our study, all within-family crosses exhibited *ID* at the larval stage (Day 9), whereas the opposite was observed for the juvenile stage (Day 45). This may be because during development, slow-growing individuals were prone to death, while the surviving individuals grew faster, and therefore, showed obvious inbreeding advantages in the juvenile stage. Taris et al. (2007) reported similar results for Pacific oysters. As the offspring grew, *ID* did not disappear but the degree was different, which may also be related

to high mortality during the larval stage. For both the selected and control groups, the *ID* of the BB group was highest during the entire experimental period, which may be related to the degree of *ID* of the parents.

Several researchers have estimated genetic parameters for growth of adult *P. martensii* after mass selection. After Wada (1986) conducted three generations of mass selection on *P. martensii*, the realized heritability values of shell length and width were 0.47 and 0.35, respectively. He et al. (2006) reported that the shell length genetic gain of the first generation was 3.91%. He et al. (2008) conducted mass selection on *P. martensii*, and the genetic gain of shell height at harvest was 15.86% and the realized heritability was 1.065. Deng et al. (2009b) found that the 360-day-old shell length genetic gain of *P. martensii* was 16.6%. Wang et al. (2011) reported that the third generation breeding population of *P. martensii* had a genetic gain of 13.27 and 13.83% for shell length and height at 360 days, respectively. On Day 360 in our study, the shell width genetic gain of *P. martensii* was in the range of 6.18–16.35%. The genetic gain of the two reciprocal hybrid crosses was larger than that of the two within-family crosses. The reason for the lower genetic gain of the two within-family crosses may be related to *ID* because the offspring were all born from a pair of parents. When harvesting, the realized heritability of shell width ranged from 0.24 to 0.70. Like genetic gain, the realized heritability of reciprocal hybrid crosses was greater than that of within-family crosses. Only the realized heritability of the SAA groups was lower than the result reported by Wada (1986), which may be because we only carried out one generation of selection. From Days 210 to 360 in our study, the realized heritability of shell width for reciprocal hybrid crosses increased, indicating that the breeding program was effective because of the significant additive genetic variation (Dégremont et al., 2015). Newkirk (1980) predicted that the genetic improvement of shellfish will be between 10 and 20% per generation. Heritability values above 0.20 indicate that genetic improvement can be easily achieved through the application of selective breeding programs (Newkirk et al., 1977; Falconer and Mackay, 1996).

In summary, we found that using two families as the base populations for mass selection successfully improved the growth performance of *P. martensii*. Although the within-family crosses produced a certain degree of *ID*, growth was improved to a certain extent. In contrast, the growth performance of reciprocal hybrid crosses improved more significantly, resulting in greater responses to selection. Therefore, the method of combining cross-breeding and mass selection is effective. Therefore, the combination of crossbreeding and mass selection is an ideal breeding method. It can provide a large number of excellent seeds for pearl industry and alleviate the shrinking status of pearl shell industry. Of course, if we want to revitalize the pearl oyster industry in China, we still need a lot of follow-up work.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

CF and XKZ designed the experiment, performed the experiments, analyzed the data, wrote the manuscript, and revised manuscript. LT designed the experiment and performed the experiments. XZZ, JL, and YL performed the experiments. QL supported site. ZW revised the manuscript. All authors contributed to the article and approved the submitted version.

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